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Subcontractor Report

Bioassay Analyses of Particulate Matter From a Diesel Bus Engine Using Various Biodiesel Feedstock Fuels

Final Report
Report 3 in a series of 6

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I. EXECUTIVE SUMMARY

Diesel exhaust is a complex mixture of compounds present in both the particle and vapor phases and includes specific classes of compounds such as the polycyclic aromatic hydrocarbons, many of which are genotoxic. Many technologies have been employed to reduce diesel particulate emissions, including engine modification, emission control devices, and the use of alternative fuels such as biodiesel. Biodiesel or biodiesel blends with diesel fuel have been studied for their potential to reduce criteria and toxic air pollutant emissions. Biodiesel fuel can be produced from both plant- and animal-based feedstocks. However, very few studies have compared emissions from biodiesel fuel derived from both of these sources, especially for particulate and toxic emissions. Therefore, additional approaches to help identify and screen for toxics present in the exhaust would be an important health assessment and evaluation tool. In the current study supported by the National Renewable Energy Laboratory (NREL), the U.S. Department of Energy (DOE), and in collaboration with the Colorado Institute for Fuels and Engine Research (CIFER), Colorado School of Mines (CSM), particulate emissions were collected and quantified from a heavy duty diesel engine using five different biodiesel fuels from various source materials.

Particulate matter was extracted and tested in a modified Salmonella/microsome bioassay described by Ames et al (1975). Dose-response curves were developed to examine the mutagen profiles from different fuel particulate matter. The mutagenic activity of each particulate sample was used to calculate a mutagen emission rate for each biodiesel fuel. The mutagen emission rate is reported as the level of mutagenic activity emitted per unit of work of the engine, or per brake horsepower-hr.

The mutagen emission rates, with and without S9 metabolic enzymes, for pork lard methyl ester (PLME), beef tallow (edible) methyl ester (BTME), and yellow grease methyl ester (YGME) were quite similar. These emission rates in general were also lower than for a certification diesel fuel (D2). Under cold start conditions, soy methyl ester (SME) appears to have the lowest mutagen emission rate compared to the other biodiesel fuels and the certification fuel. Of the five biodiesel emission samples collected under cold start conditions, SME had the lowest mutagen emission rate (1.19×10^5 Rev/BHP-HR) and canola methyl ester (CME) had the highest rate (3.76×10^5 Rev/BHP-HR) when tested in the bioassay without the addition of metabolic enzymes.

The mutagen emission rates for the biodiesel fuels are less than one-half the rate for D2 (4.52×10^5 Rev/BHP-HR), with the exception of CME which had an emission rate close to the D2 fuel and a rate that was higher then the other biodiesel fuels. Under hot start conditions, beef tallow (edible) methyl ester and yellow grease methyl ester appear to have the lowest mutagen emission rate. For mutagen emissions under hot start conditions and tested without metabolic enzymes (-S9), BTME (edible) had the lowest mutagen emission rate (1.59×10^5 Rev/BHP-HR), while PLME and SME had the highest rates. The use of biodiesel fuels from different source materials can reduce the emissions of particle-bound toxic mutagenic compounds.

II. INTRODUCTION

Diesel engine exhaust is a complex mixture of compounds that is composed of both gaseous and particulate matter (PM) emissions. Some of these compounds include known and unknown genotoxic compounds. Since PM is a federally regulated emission, alternative technologies and fuels have been developed and used to reduce the emissions of PM from diesel engines. The use of biodiesel alone or the addition of biodiesel to diesel fuel can substantially reduce PM emissions from diesel engines. Biodiesel can be produced from both plant and animal feedstocks. A typical source of biodiesel is the oil from safflower, rapeseed, canola, and soybean plants. Biodiesel can also be produced from other sources, including animal lard. To examine the effect of using biodiesel produced from different feedstocks on PM emisions, the Colorado Institute for Fuels and Engine Research, Colorado School of Mines (CSM) has collected emission samples from one engine using seven different biodiesel fuels that were produced from various source materials. The regulated emissions (NOx, CO, THC, and PM) were determined by CSM, as well as the volatile organic fraction of PM (NREL report NREL/SR-510-31461). In collaboration with researchers at the University of California at Davis, representative samples from diesel and biodiesel particulate emissions were tested for genotoxicity.

The unidentified compounds present in these complex mixtures can be important in the exposure of the population to toxic compounds. Therefore, a supplemental approach to help identify and screen for toxic compounds present in the complex mixture of diesel exhaust would be an important assessment tool.

One approach to screening and identifying potentially toxic compounds is to use a bioassay. Although there have been many studies on diesel PM and its genotoxic activity, fewer studies have been conducted on toxic emissions from the use of biodiesel as a fuel. In a typical experiment, PM is extracted using organic solvents, concentrated by evaporation, and the extracts are then individually tested in a bioassay. The bioassay used at U.C. Davis is a microsuspension modification (Kado et al., 1983) of the Salmonella/ microsome test of Ames et al. (1975). Using the Kado test, the mutagenic activity of each extract is obtained and the results can be used to compare mutagenic activities and compound profiles from different sources, locations, or collection variables.

In the present study, emission samples were collected by CSM personnel from one engine using five different biodiesel fuels that were produced from various source materials. Selected samples were processed at UC Davis, tested in the bioassay, and the mutagen emission rates were calculated. These rates are reported as the level of mutagenic activity per unit of work of the engine, or bacterial revertants per brake horsepower-hour.

III. MATERIALS AND METHODS

A. Test Fuels

The biodiesel fuels tested are listed in Table 1. They are derived from plant (canola, soy) or animal (beef tallow, pork lard) feedstocks and were tested without dilution (neat) in a diesel engine. For each fuel type, particulate emission samples were collected for one cold start and three hot starts. The test sequence for each fuel is detailed in a report by McCormick et al. (1999).

Table 1. Test fuel and type of samples collected for bioassay analyses

Test Fuel	Abbreviation	Test Type
Soy Methyl Ester	S M E	1 Cold, 3 Hot
Canola Methyl Ester	C M E	1 Cold, 3 Hot
Pork Lard Methyl Ester	P L M E	1 Cold, 3 Hot
Beef Tallow (Edible) Methyl Ester	B T M E	1 Cold, 3 Hot
Yellow Grease Methyl Ester (Low Free Fatty Acid, 1%)	Y G M E	1 Cold, 3 Hot
Phillips Certification	D2	1 Cold, 6 Hot

A diesel certification fuel (D2) was also tested for purposes of comparison. This reference fuel was obtained from Phillips Petroleum. The properties of the certification and biodiesel fuels are shown in Table 2 (McCormick et al., 1999). In addition, one blank sample from the sampling system was also collected.

Table 2. Test fuel properties[a]

Fuel	Carbon wt%	Hydrogen wt%	Oxygen wt%	Cetane Number	Heat of Combustion (btu/lb)
D2[b]	86.6	13.4	0	46.0	18456
S M E	76.25	12.59	11.16	47.2[c]	17130
C M E	76.12	12.84	11.04	55.0	17074
P L M E	75.03	13.15	11.82	63.6	17084
B T M E	75.15	13.11	11.74	62.9	17120
Y G M E	75.71	13.19	11.10	57.8	17133

[a] as reported by McCormick et al., 1999.

[b] sulfur content = 300 ppm, aromatics vol% = 29.2.

[c] IGT reported a cetane number of 59.

B. Test Engine and Facility

Particulate emission samples for each test fuel were collected from a 1991 production model Detroit Diesel 6-cylinder 4-stroke engine. The engine was electronically controlled, direct injected, turbocharged, and had intercooled calibration. The test engine specifications are listed in Table 3.

Table 3. Test engine specifications [a]

Make	Detroit Diesel Corporation (DDC)
Model	DDC Series 60
Serial number	6R-544
Year	1991
Displacement	11.1 Liters
Cylinders	6
Horsepower/Rated speed	345 BHP @ 1800 rpm

[a] as reported by McCormick et al., 1999.

Emissions testing was conducted at the Colorado Institute for Fuels and Engine Research (CIFER), Colorado School of Mines (CSM), located in Golden Colorado. The engine test cell consisted of a DC dynamometer, in-line and reaction torque cells, with in-line speed pickup. This facility is equipped with two eighteen-inch stainless steel dilution tunnels. A rooftop unit equipped with filters, a humidification system, and blower were used to condition the dilution and engine air. The tunnel flowrate was measured using a critical flow venturi device with inlet temperature and pressure monitoring. For these engine tests, researchers used a 2300 scfm Venturi.

C. Sample Collection

Specific details of emissions testing and the particulate sample collection procedure are outlined in the final report by McCormick et al. (1999). Briefly, particulate emission samples for each test fuel were collected from a secondary dilution tunnel at temperatures below 52°C using two independent mass flow controllers to regulate the total filtered gas sample and secondary dilution air rate. Both intake and supply air were conditioned. The emissions testing system met all requirements for heavy-duty engine emissions certification testing as listed in the Code of Federal Regulations 40, Part 86, Subpart N.

The particulate matter was collected on pre-cleaned 70 mm Teflon-containing glass fiber filters (T60A20, Gelman Sciences-Pallflex). The filters were conditioned and weighed under yellow lights in a constant humidity weigh room held at 9 ± 2°C (48 ± 4°F) dew point, 50% relative humidity and 22 ± 1°C (72 ± 2°F). After sample collection, the filters were conditioned and weighed using a five digit electronic balance. Filter samples were carefully packaged and shipped to the U.C. Davis laboratory and stored in the freezer at –20°C until extracted.

D. Sample Extraction

Prior to extraction, the filters were removed from the freezer and allowed to equilibrate to room temperature. The identity of each filter sample was verified and recorded. Dichloromethane (DCM)-rinsed tweezers and spatulas were used to carefully remove the sample filters from glassine storage envelopes. For the biodiesel emission samples, whole filters were extracted to provide sufficient mass of particles for bioassay testing. For the D2 emission samples, one half of the filter was extracted for bioassay testing and the other half was archived. The weight of each filter half was recorded.

All glassware used for the filter extractions was solvent-rinsed with methanol and DCM. The filter pieces were placed sample face up into a screw-top flask and DCM was added. Filters were sonicated for 15 minutes and the sonicator bath temperature was maintained between 22-30°C. After sonication, each extract was transferred to a separate labeled "holding" flask and the procedure was repeated two more times. The sample extracts were filtered using a filter unit (0.45µm pore size, Gelman CR PTFE), concentrated to 0.5 mL by nitrogen evaporation, and the evaporation vessel was rinsed with 0.5 ml DCM, and placed into a pre-cleaned amber vial. After the final weight of each amber vial was recorded, the sample extracts were stored in the freezer at –20°C until testing in the bioassay.

E. Bioassay Testing

For the bioassay, benzo(a)pyrene was obtained from Sigma Chemical Co. (St. Louis, MO). Dimethyl sulfoxide (DMSO, ACS spectrophotometric grade) was purchased from Aldrich Chemical Co. (Milwaukee, WI). Fresh dilutions of the filter sample extracts were prepared in DMSO immediately prior to each mutagenicity experiment.

A microsuspension procedure previously reported by Kado et al., (1983; 1986), which is a simple modification of the Ames test, was used throughout. Tester strain TA98 was kindly provided by Dr. B.N. Ames, Berkeley, CA. For the Kado procedure, bacteria were grown overnight in Oxoid Nutrient Broth No. 2 and harvested by centrifugation. Cells were re-suspended in ice-cold phosphate-buffered saline (PBS) to a concentration of approximately 1 x 10^{10} cells/ml. The S9 (metabolic enzymes) and S9 mix (enzyme co-factors) were prepared according to the procedure of Ames et al., (1975). The S9 from Aroclor 1254 pre-treated male Sprague-Dawley rats was obtained from Molecular Toxicology, Inc. (Annapolis, MD) and contained 40 mg protein/ml, as determined using the method of Lowry et al., (1951).

For each bioassay experiment conducted, the following ingredients were added, in order, to sterile glass culture tubes kept on ice: S9 mix or cocktail, concentrated bacteria in PBS, and the test sample. Filter sample extracts were tested at three different concentrations, in duplicate, based on the original amount of PM collected on the filter (extracted microgram PM equivalent). These concentrations were chosen based on range finding experiments (data not shown). One sampling system blank and three laboratory extraction blanks were also tested. The mixture was incubated in the dark at 37°C with rapid shaking. After 90 minutes, the tubes were placed in an

ice bath and taken out one at a time immediately before adding molten top agar containing histidine and biotin (Ames et al., 1975). The combined solutions were vortex-mixed and poured onto minimal glucose plates. Plates were incubated at 37°C in the dark for 48 hours and counted. Benzo(a)pyrene and 2-nitrofluorene were used as positive controls. Strain markers were routinely determined for each experiment.

IV. RESULTS AND DISCUSSION

A. Particle Emissions

Particle emission rates for the single cold starts and the multiple hot starts were provided by CSM for all filter samples that were tested in the bioassay. Along with the bioassay results, this data was used to calculate emission rates for total mutagenic activity from the engine exhaust and the use of a particular test fuel. For regulatory reporting purposes, cold and hot start runs are typically combined in a weighted average (1/7 cold + 6/7 hot). In this study, a weighted average for composite emissions could not be calculated since only three hot start filter samples were provided for each fuel that was tested.

The particle emission rate for the certification fuel under cold start conditions was approximately three times higher than the rates for the five biodiesel fuels that were tested. The particle emission rates were provided by CSM (McCormick et al., 1999) and the data is shown in Table 4. Of the five biodiesel emission samples collected under cold start conditions, BTME (edible) had the lowest PM emission rate and CME had the highest rate. However, the range of emission rates for the biodiesel fuels was from 0.76 to 0.103 g/BHP-HR, while the emission rate for D2 was 0.430 g/BHP-HR. The ranking of PM emission rates for the fuels tested was as follows (from highest to lowest): D2 > CME > SME = PLME > YGME (LFFA) > BTME (edible). This ranking is provided as a relative comparison only and is not intended as a definitive comparison of PM emission rates for these fuels.

Table 4. Particulate emission rate for single cold starts

Test Fuel	U.C. Davis Sample ID	CSM Run No.	BHP-HR[a]	PM g/BHP-HR[b]
D 2	CbioD.5	4517	22.399	0.340
S M E	CbioD.9	4524	22.298	0.093
C M E	CbioD.13	4532	22.403	0.103
P L M E	CbioD.17	4539	22.231	0.092
B T M E (edible)	CbioD.21	4569	22.039	0.076
Y G M E	CbioD.25	4585	22.081	0.083

[a] brake horsepower-hour

[b] grams of particulate matter per brake horsepower-hour

The particle emission rate for D2 under hot start conditions was also substantially higher than the rates for the five biodiesel fuels tested. The particle emission rates were determined by CSM as summarized in Table 5. Of the five biodiesel emission samples collected under hot start conditions, YGME (LFFA) and PLME had the lowest PM emission rates and SME had the highest rate, although these values were fairly similar. The range of emission rates for the biodiesel fuels was from 0.068 to 0.081 g/BHP-HR.

Table 5. Particulate emission rate for multiple hot starts

Test Fuel	UC Davis Sample ID	CSM Run #	Mean PM g/BHP-HR[a]	Std. Dev.
D 2-1[b]	CbioD.6 - 8	4518-20	0.309	(± 0.108)
S M E	CbioD.10-12	4525 – 27	0.081	(± 0.004)
C M E	CbioD.14-16	4533-35	0.077	(± 0.004)
P L M E	CbioD.18-20	4540-42	0.068	(± 0.004)
B T M E	CbioD.22-24	4570-72	0.070	(± 0.002)
Y G M E LFFA	CbioD.26-28	4586-88	0.068	(± 0.002)
D 2-2[c]	CbioD.29	4546-7 4560	0.268	(± 0.010)

[a] grams of particulate matter per brake horsepower-hour

[b] D2 fuel tested before biodiesel fuels. One cold start was run prior to this test.

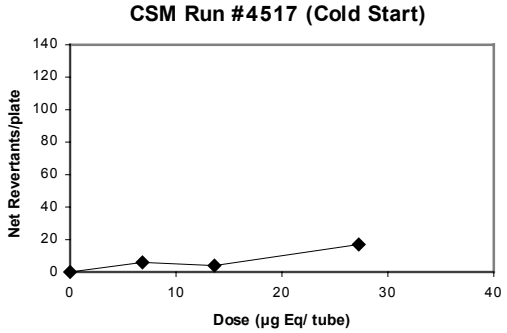
[c] D2 fuel tested after testing the biodiesel fuels. Runs 4546-7 were consecutive; run 4560 was conducted after five D2 Hot starts (4550-4554; runs 4548-9 are unaccounted for) and three Inedible Tallow hot starts (4555-4557; runs 4558-9 are unaccounted for) .

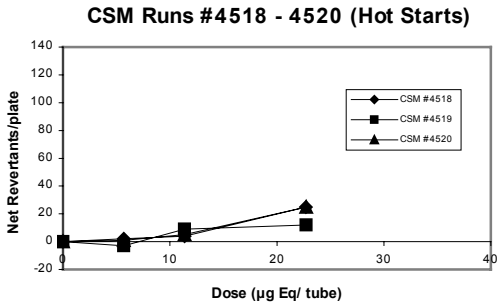
The ranking of PM emission rates for the fuels that were tested was as follows (from highest to lowest): D2 (before testing biodiesel fuels) > D2 (after testing biodiesel fuels) > S M E > C M E > B T M E (edible) > P L M E = Y G M E (LFFA). This ranking is also provided as a relative comparison only and is not intended as a definitive comparison of PM emission rates for these fuels. For all fuels tested, the PM emission rate under hot start conditions was lower than the rate under cold start conditions.

B. PM Specific Activity – Mutagenic Potency

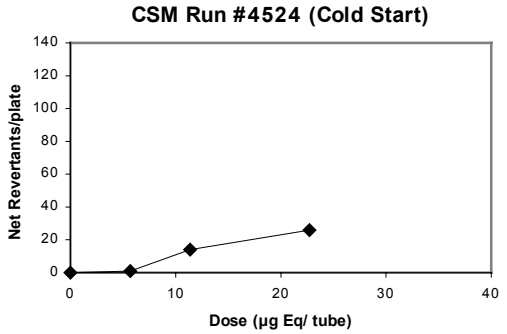
The PM that was collected from the use of each test fuel was extracted and tested in the bioassay, with and without the addition of metabolic enzymes (±S9). For all test fuels, the PM that was collected under both cold and hot start conditions was mutagenic in the bioassay. Extraction blank samples were tested but were not mutagenic (data not shown), indicating that the extraction procedure and filter material did not contribute to the mutagenicity of the PM emission samples. The bioassay results were used to create dose-response curves for each filter sample. Dose-response curves for the cold and hot start tests using D2 and biodiesel fuels representative of plant (S M E) and animal (B T M E) sources are presented in Figures 1 and 2 for +S9 and –S9, respectively. Using the slope from the linear portion of each dose-response curve, a mutagenic potency value was calculated for each PM sample. The mutagenic potency values for each test fuel under cold start and hot start conditions are shown in Tables 6 and 7, respectively. These values represent the number of TA98 revertants per mass of particles collected, and are expressed as "revertants per microgram equivalent." The term "equivalent" refers to the amount of extract added to the test system that is representative of the mass of PM.

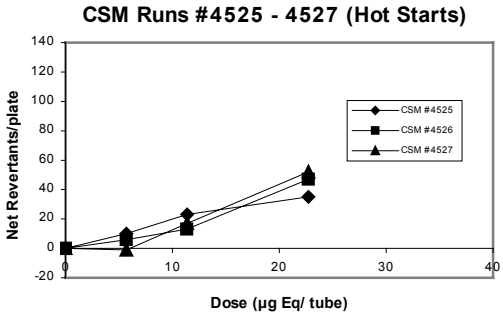
Certification Fuel (D2)



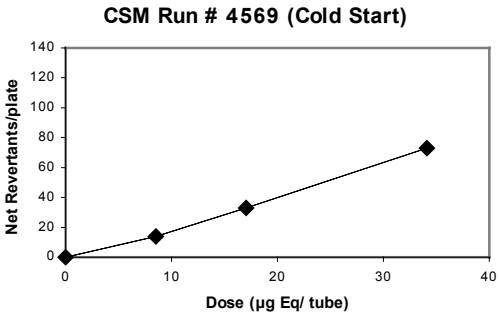


Soy Methyl Ester (SME)





Beef Tallow Methyl Ester (BTME)



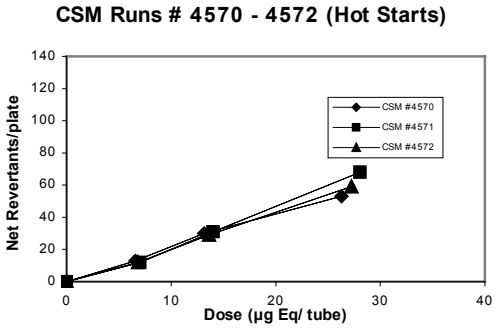
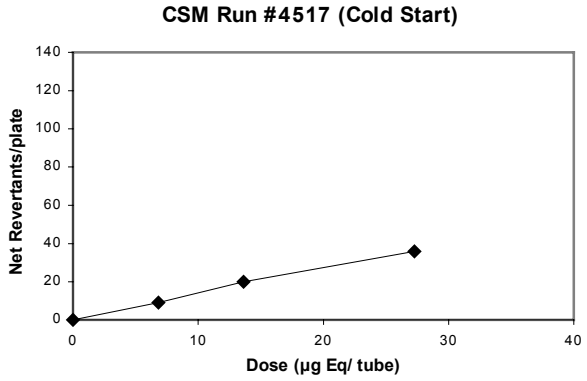
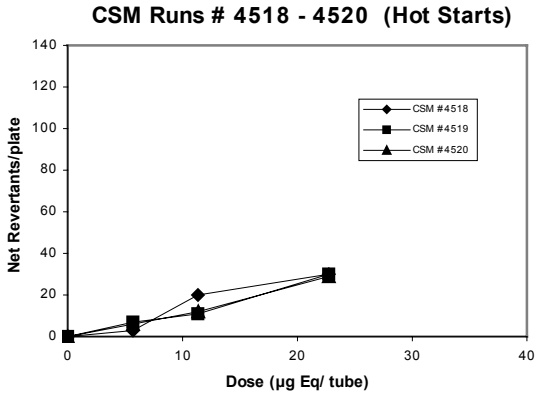


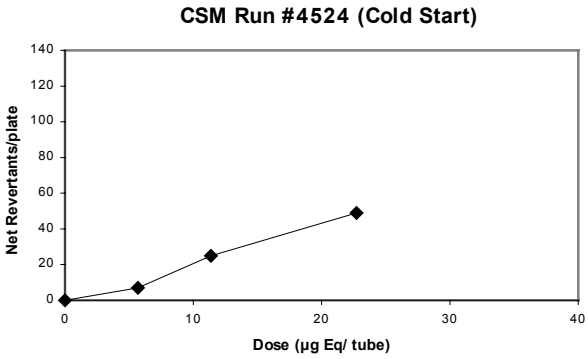
Figure 1. Dose-response curves for D2, SME, and BTME fuels with tester strain TA98 with metabolic enzymes added (+S9). The SME and BTME are representative of plant (SME) and animal (BTME)-derived biodiesel fuels.

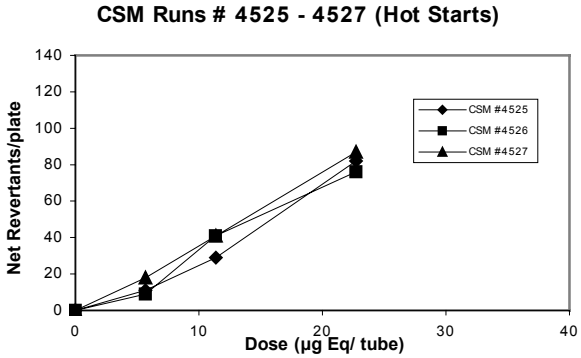
Certification Fuel (D2)



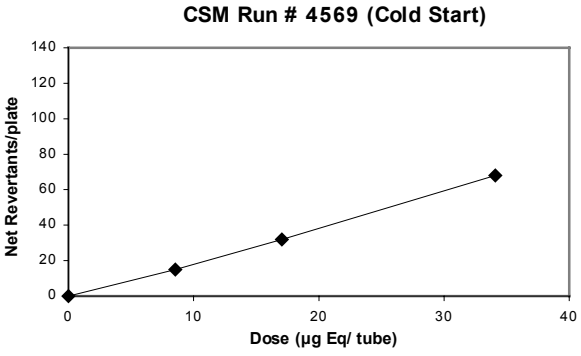


Soy Methyl Ester (SME)





Beef Tallow Methyl Ester
(BTME)



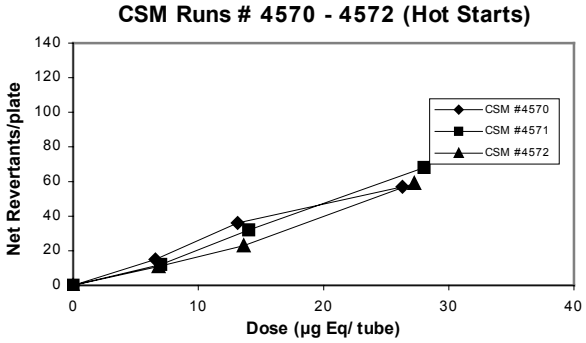


Figure 2. Dose-response curves for D2, SME, and BTME fuels with tester strain TA98 without metabolic enzymes added (-S9). The SME and BTME are representative of plant (SME) and animal (BTME)-derived biodiesel fuels.

Table 6. Mutagenic potency values for test fuels - cold start conditions.

Test Fuel	UC Davis Sample ID	CSM Run #	TA98 Mutagenic Potency (Rev/µg Eq)* (+S9)	(-S9)
D 2	CbioD.5	4517	0.84**	1.32**
S M E	CbioD.9	4524	1.23	1.28
C M E	CbioD.13	4532	2.53**	2.82**
P L M E	CbioD.17	4539	2.23	1.69
B T M E (edible)	CbioD.21	4569	2.17	2.01
Y G M E (LFFA)	CbioD.25	4585	2.69	2.04

* Data for each fuel represents TA98 revertants per microgram equivalent from a single cold start; +S9 = bioassay with metabolic enzymes, -S9 = bioassay without metabolic enzymes.
**Data is the average result from two independent bioassay experiments.

The mutagenic potency values for the three hot starts for each fuel were very close statistically, with an average relative percent standard deviation of 17.3 and 10.8 for +S9 and –S9, respectively. The mutagenic potencies for the biodiesel fuels, under both cold and hot start conditions, were higher than for the D2 fuel. This indicates that there was more mutagenic activity per particle mass for the biodiesel fuels. However, to evaluate potential real-world exposures to mutagenic compounds, the emission rate of PM for each fuel is incorporated into the calculation of mutagen emissions. These emission values are presented and discussed in the section that follows.

Table 7. Mutagenic potency values for test fuels - hot start conditions

Test Fuel	UC Davis Sample ID	CSM Run #	Mean TA98 Mutagenic Potency (Rev/µg Eq)[a] (+S9)	Std. Dev.	(-S9)	Std. Dev.
D 2-1[b]	CbioD.6-8	4518-20	0.99	(± 0.31)	1.32	(± 0.04)
S M E	CbioD.10-12	4525-27	2.03	(± 0.45)	3.69	(± 0.18)
C M E	CbioD.14-16	4533-35	1.72	(± 0.25)	2.99	(± 0.13)
P L M E[c]	CbioD.18-20	4540-42	3.66	(± 0.33)	3.83	(± 0.32)
B T M E	CbioD.22-24	4570-72	2.23	(± 0.22)	2.29	(± 0.17)
Y G M E LFFA	CbioD.26-28	4586-88	2.59	(± 0.32)	2.61	(± 0.12)
D 2-2[d]	CbioD.29	4546-7, 4560	1.16	(± 0.14)	1.15	(± 0.41)

[a] Data for each fuel represents mean TA98 revertants per microgram equivalent from 3 consecutive hot start runs.

[b] D2 fuel tested before biodiesel fuels. One cold start was run prior to this test. Data for CSM Run # 4518 is the average of 3 independent bioassay experiments.

[c] Data for CSM Run #4540 is the average of 2 independent bioassay experiments.

[d] D2 fuel tested after testing the biodiesel fuels. Runs 4546-7 were consecutive; run 4560 was conducted after five D2 Hot starts.

+S9: bioassay with metabolic enzymes; -S9: bioassay without metabolic enzymes.std dev: standard deviation.

C. Mutagen Emission Rates

The mutagenic potency value and particle emission data were both used to calculate an emission rate for mutagens in the engine exhaust from the use of a particular test fuel. Mutagen emission rates were calculated for the single cold start and multiple hot starts for all PM emission samples tested in the bioassay.

Based on the bioassay results for TA98 without S9 metabolic enzymes, the mutagen emission rate with D2 under cold start conditions was higher than the rates from the five biodiesel fuels that were tested, as shown in Table 8. Of the five biodiesel emission samples collected under cold start conditions SME had the lowest mutagen emission rate (1.19×10^5 Rev/BHP-HR) and CME had the highest rate (3.76×10^5 Rev/BHP-HR) when tested in the bioassay without the addition of metabolic enzymes. The mutagen emission rates for the biodiesel fuels are less than one-half the rate for D2 (4.52×10^5 Rev/BHP-HR), with the exception of CME, which had an emission rate close to that of the D2 fuel and a rate that was higher then the other biodiesel fuels. This data is also presented graphically in Figures 3 and 4. Under cold start conditions, the mutagen emission rate for D2 with the addition of S9 metabolic enzymes was similar to the rate for CME, but was higher than the rates for SME, PLME, BTME (edible), and YGME.

Following a single cold start test, there were three separate hot start emission samples collected for each test fuel. Hot start mutagen emission rates calculated for each test fuel represent an average of the three consecutive tests. Mutagen emission rates were calculated for D2 samples that were acquired before and after the five biodiesel fuels were tested. Following 25 cycles of testing the biodiesel fuels, there was a very slight increase in the mutagen emission rate for D2 tested with metabolic enzymes (+S9) and a slight decrease in the mutagen emission rate for D2 tested without metabolic enzymes (-S9). The hot start mutagen emission rates for D2 (±S9) were higher than the rates from the five biodiesel fuels that were tested, as summarized in Table 9. The CME emissions (1.33×10^5 Rev/BHP-HR) were the lowest of all biodiesel fuels tested and PLME emissions (2.49×10^5 Rev/BHP-HR) were the highest. For mutagen emissions under hot start conditions and tested without metabolic enzymes (-S9), BTME (edible) had the lowest mutagen emission rate (1.59×10^5 Rev/BHP-HR), while PLME and SME had the highest rates. This data is also presented graphically in Figures 5 and 6.

Table 8. Mutagen emission rate for test fuels - cold start conditions

Test Fuel	UC Davis Sample ID	CSM Run #	BHP-hr	Mutagen Emission Rate (Revertants / BHP-HR x 10^5)* TA98 (+S9)	TA98 (-S9)
D 2	CbioD.5	4517	22.399	2.86	4.49
S M E	CbioD.9	4524	22.298	1.14	1.19
C M E	CbioD.13	4532	22.403	2.61	2.90
P L M E	CbioD.17	4539	22.231	2.05	1.55
B T M E (edible)	CbioD.21	4569	22.039	1.65	1.53
Y G M E (LFFA)	CbioD.25	4585	22.081	2.23	1.69
SME-C [b]	(H412,H435,H444)	NA		1.82	2.28
D2-C [b]	C570	NA		9.9	10.49

* Data for each fuel represents mean TA98 revertants per brake horsepower-hour from a single cold start; +S9 = bioassay with metabolic enzymes and -S9 = bioassay without metabolic enzymes.

[b] SME and D2 fuel tested at Caterpillar technical center (Mossville, Il.) on a Cat 3406E engine.
NA = not applicable.

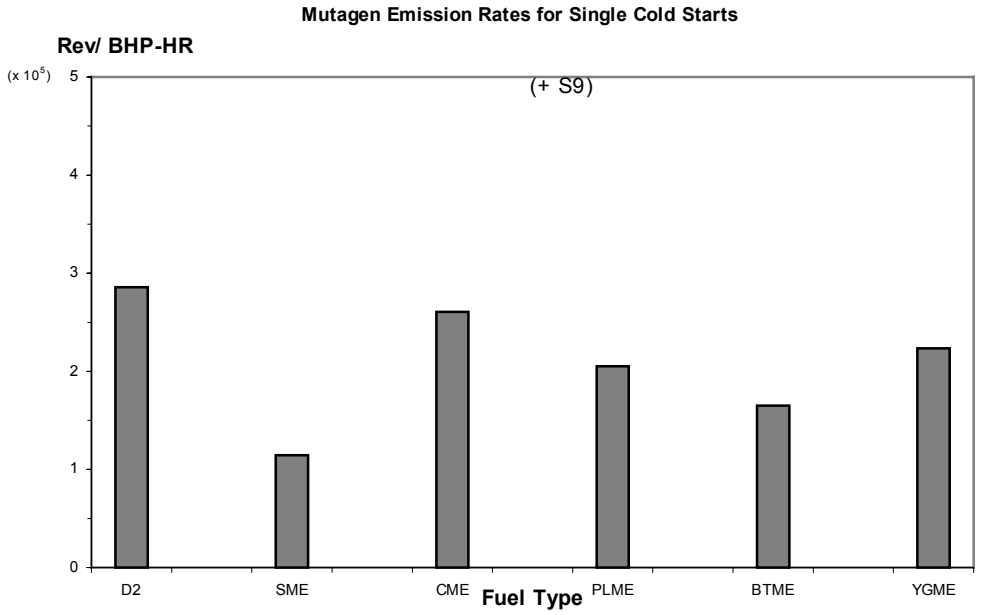


Figure 3. Cold Start Mutagen Emission Rates for Different Test Fuels (TA98 +S9)

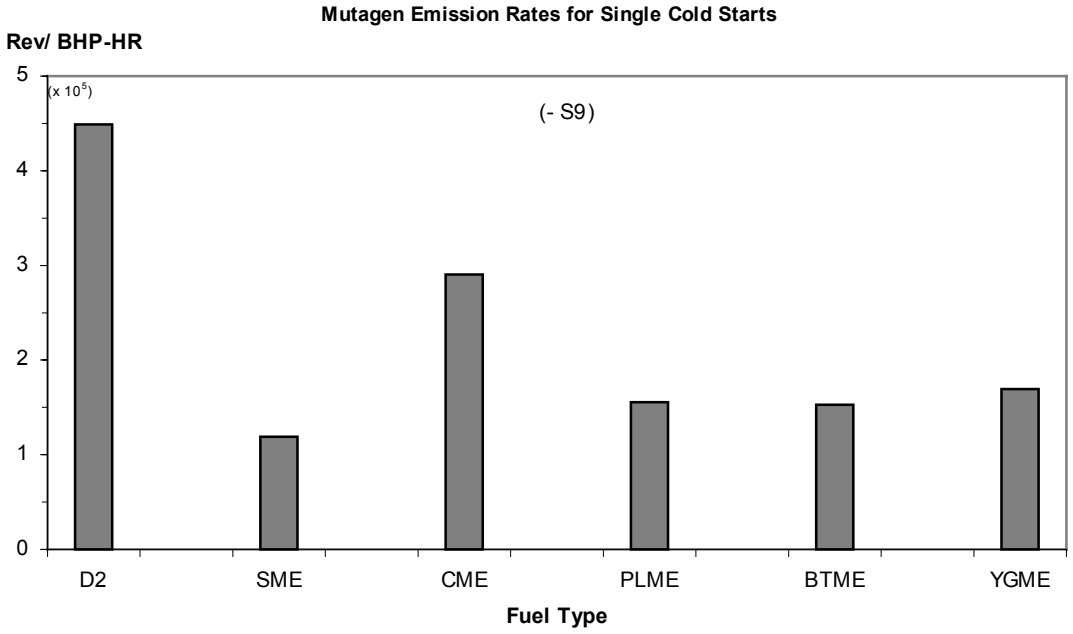


Figure 4. Cold Start Mutagen Emission Rates for Different Test Fuels (TA98 –S9)

Table 9. Mutagen emission rate for test fuels - hot start conditions

Test Fuel	UC Davis Sample ID	CSM Run #	Mutagen Emission Rate (Revertants / BHP-HR x 10^5)* TA98 (+S9)	Std. Dev.	TA98 (-S9)	Std. Dev.
D 2-1[a]	CbioD.6-8	4518-20	3.05	(± 0.945)	3.93	(± 0.292)
S M E	CbioD.10-12	4525-27	1.66	(± 0.437)	2.99	(± 0.260)
C M E	CbioD.14-16	4533-35	1.33	(± 0.237)	2.29	(± 0.015)
P L M E	CbioD.18-20	4540-42	2.49	(± 0.177)	2.62	(± 0.284)
B T M E	CbioD.22-24	4570-72	1.56	(± 0.187)	1.59	(± 0.146)
Y G M E LFFA	CbioD.26-28	4586-88	1.77	(± 0.269)	1.78	(± 0.114)
D 2-2[b]	CbioD.29	4546-7, 4560	3.10	(± 0.264)	3.05	(± 0.996)
SME-C [c]	(H412,H435,H444)	NA	2.65	(± 0.13)	3.89	(± 0.28)
D2-C [c]	(H550, H560, H574)	NA	8.23	(± 1.91)	9.42	(± 1.34)

* Data for each fuel represents mean TA98 Revertants/BHP-HR from 3 consecutive hot runs.
+S9: bioassay with metabolic enzymes. -S9: bioassay without metabolic enzymes. std dev:standard deviation
[a] D2 fuel tested before biodiesel fuels.
[b] D2 fuel tested after testing the biodiesel fuels.
[c] For comparison, SME and D2 fuels were tested at Caterpillar technical center (Mossville, Il.) on a Cat 3406E 14.6 L diesel engine. The test cycle was the EPA Heavy-Duty diesel engine transient test cycle (CFR40, Pt.86, Subpt. N). SME fuel was from C. Peterson (U. Idaho) and D2 was certification fuel (Phillips Petroleum). NA: not applicable.

The hot start mutagen emission rates, with and without S9 metabolic enzymes, for PLME, BTME (edible), and YGME were quite similar, whereas SME and CME had greater emissions without metabolic enzymes. This may indicate that different mutagenic compounds are emitted with biodiesel fuels derived from plant- and animal-based feedstocks. These compounds may be nitro-polyaromatic hydrocarbons, but confirmation would require chemical analysis of these samples. The mutagenic potency (revertants/microgram) and mutagen emission rate for D2 appear somewhat lower than for previous samples tested in this lab for other diesel engines.

The U.C. Davis lab had previously tested SME and D2 fuel in a 1997 Caterpillar diesel engine (14.6 L, Model 3406E). The mutagen emission rates for SME collected under hot start conditions in this previous study averaged 2.65 and 3.89 x 10^5 Rev/BHP-HR, for +S9 and –S9 treatments, respectively, as seen in Table 9 (Kado et al., 1998). The emissions rates for the SME fuel for the current study are 1.6 and 1.3 times lower (+S9 and –S9, respectively). For the D2 fuel tested in the previous study, the emission rates averaged 8.2 and 9.4 x 10^5 Rev/BHP-HR (+S9 and –S9, respectively) as seen in Table 9. In the current study and using the same D2 certification fuel, the mean emission rate was 3.07 and 3.49 x 10^5 Rev/BHP-HR for +S9 and –S9, respectively. These current emission rates are approximately 3 times lower than in the previous study. However, for both SME and D2, the emission rates overall were in the same order of magnitude of 10^5 Rev/BHP-HR. The lower emissions from the current study may be due to differences in engine design and testing at different environmental conditions.

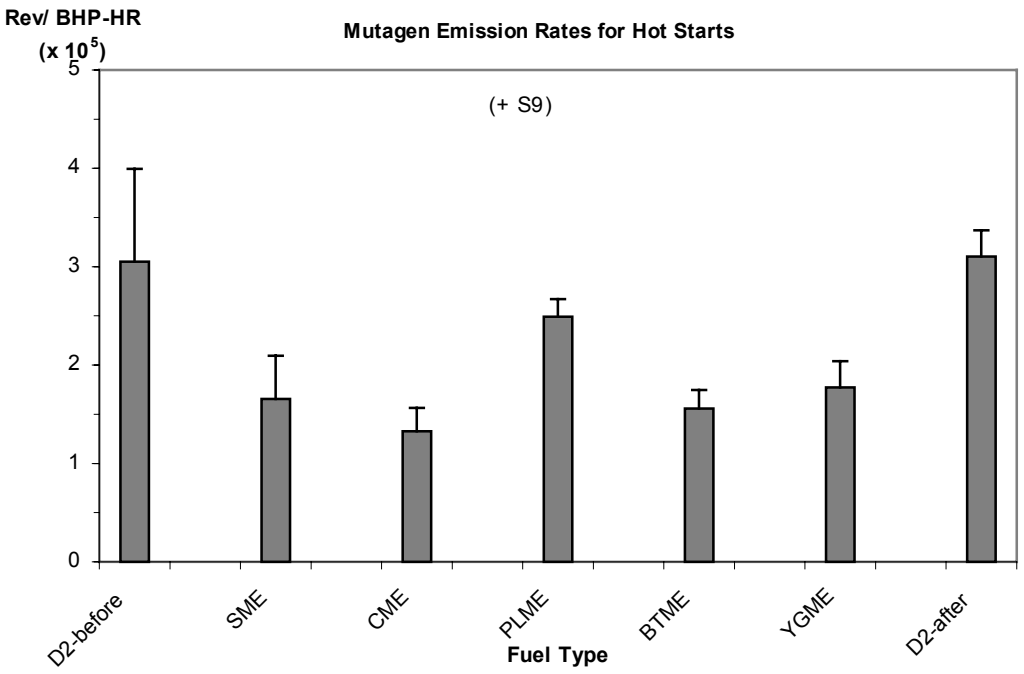


Figure 5. Hot Start Mutagen Emission Rates for Different Test Fuels (TA98 +S9)

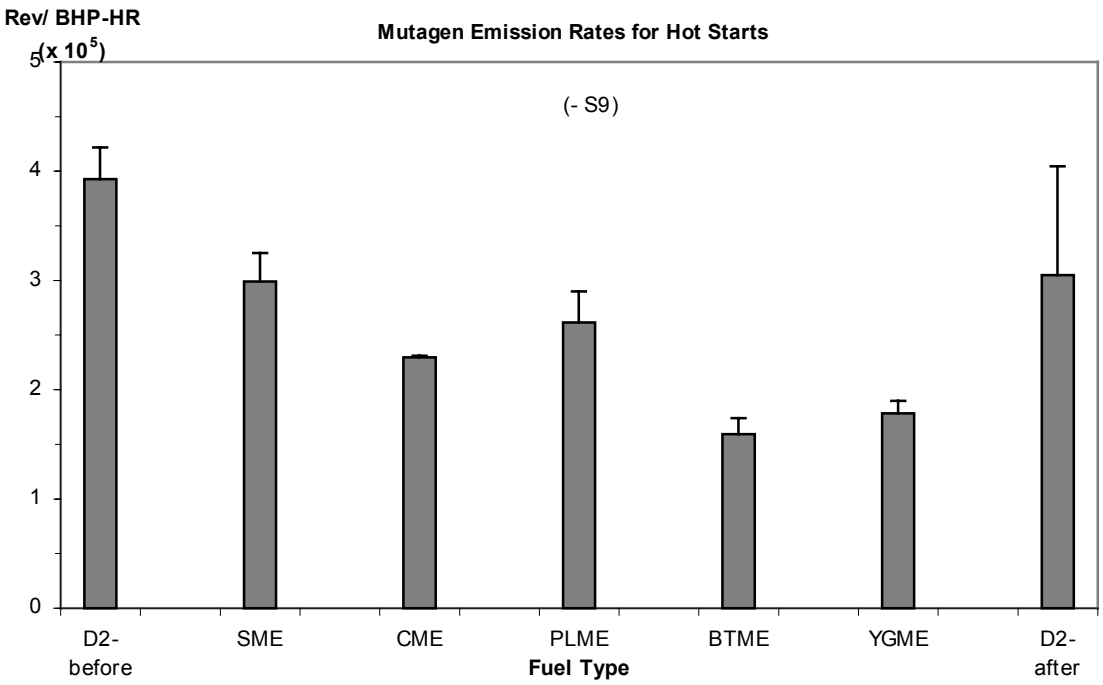


Figure 6. Hot Start Mutagen Emission Rates for Different Test Fuels (TA98 –S9)

Emissions testing at higher altitudes has been reported to increase particulate emissions and to decrease the percentage of soluble organic fraction relative to soot, when compared to testing an identical engine with the same fuel at sea level (Graboski and McCormick, 1996).

Few studies have reported on the mutagenic activity of biodiesel emissions. Bünger et al (1998) examined the mutagenic and cytotoxic effects of emissions from a diesel car using rapeseed methyl ester (RME) as a biodiesel fuel and compared the results to those obtained using conventional diesel fuel. In general, the RME biodiesel fuel emissions were substantially less mutagenic than the diesel emissions on a per milligram particulate and per kilometer basis. In addition to the Salmonella mutagenicity studies, the authors examined toxicity of the filter extracts to an established cell line of mouse lung fibroblasts (L929). The extracts had increasing toxicity to the cells with increasing concentration. However, no significant differences were observed between the fuels. In a separate study, Bagley et al., (1998) examined the use of soy methyl ester (SME) biodiesel fuel on emissions from an indirect injection diesel engine, similar to those used in underground mining operations. The authors observed that using an oxidative catalytic converter resulted in over 50% reductions in both particle and vapor-phase-associated mutagenicity with both D2 and SME biodiesel fuels. When SME was used with the oxidative catalytic converter, no vapor-phase mutagenicity was observed.

The use of biodiesel fuels from different source materials can reduce emissions of particle-bound toxic mutagenic compounds. The current report is one of the initial bioassay studies comparing biodiesel fuels derived from plant and animal feedstocks with conventional diesel fuel. Further biological and chemical studies of the vapor-phase as well as particle phase are recommended to characterize and examine the toxic chemical components of biodiesel emissions from these different source materials.

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VI. GLOSSARY OF TERMS AND ABBREVIATIONS

BHP-HR	Brake horsepower-hour
BTME	Beef tallow (edible) methyl ester
CIFER	Colorado Institute for Fuels and Engine Research
CME	Canola methyl ester
CSM	Colorado School of Mines
D2	Phillips certification fuel
DCM	Dichloromethane
DOE	Department of Energy
EPA	Environmental Protection Agency
IARC	International Agency for Research on Cancer
LFFA	Low free fatty acid
NREL	National Renewable Energy Laboratory
PLME	Pork lard methyl ester
PM	Particulate matter
S9	Metabolic enzymes
SME	Soy methyl ester
TA98	Bacterial tester strain
YGME	Yellow grease methyl ester

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In this study, particulate matter (PM) emissions were collected and quantified from a heavy duty diesel engine using five different biodiesel fuels from various source materials.

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